SHIMALACTONES, NEURITOGENIC POLYKETIDES FROM A MARINE-DERIVED FUNGUS EMERICELLA VARIECOLOR GF10

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Abstract – Shimalactone B (2), a novel polyketide having bicyclo[4.2.0]octadiene and oxabicyclo[2.2.1]heptane units, was isolated as a minor constituent from a cultured marine-derived fungus of Emericella variecolor GF10. The chemical structure of 2 and the structural relationship between shimalactones A and B (2) were determined by detailed NMR and CD spectroscopic analyses and chemical derivatization. Shimalactones A and B (2) induced neuritogenesis against neuroblastoma Neuro 2A cells at 10 µg/mL concentration.

INTRODUCTION
In recent years, marine microorganisms have been paid much attention as a significant source in the search for new drug leads.1-3 As a part of our ongoing search for new biologically active substances from marine microorganisms, we previously reported the isolation of ophiobolins-type cytotoxic sesterterpenes4 and a novel polyketide named shimalactone A (1)5 from the marine-derived fungus, Emericella variecolor GF10. Shimalactone A (1) possessed a unique carbon skeleton such as bicyclo[4.2.0]octadiene and oxabicyclo[2.2.1]heptane units, of which the stereostructure was deduced from 2D NMR and X-Ray crystallographic analyses of 1 and its derivatives. Continued study on the extract of E. variecolor GF10 has led us to isolation of shimalactone B (2) as a minor constituent. Details of the structure elucidation of shimalactone B (2) are presented here.

RESULTS AND DISCUSSION
The fungus strain of Emericella variecolor GF10 was cultured for 2 weeks at 30 °C on a solid rice-based medium prepared with artificial seawater. The MeOH extract was fractionated by silica gel column chromatography and HPLC to obtain a new polyketide named shimalactone B (2) together with shimalactone A (1).
Shimalactone B (2) was obtained as a colorless oil. Its molecular formula C_{29}H_{40}O_{4} was determined by HRFABMS and NMR spectral analysis. The 1H NMR spectrum of 2 indicated the presence of eight singlet-methyl protons (δ_H 1.77, 1.66, 1.57, 1.50, 1.32, 1.15, 1.13, 0.98), two doublet-methyl protons (δ_H 1.58, 1.21), and nine methine protons (δ_H 5.46, 5.42, 5.28, 5.12, 4.76, 3.66, 3.03, 2.77, 2.68), which were closely similar to those of 1. A careful analysis of its 2D NMR (COSY, HMOC, HMBC, NOESY) spectral data resulted in the conclusion that compound (2) has the same planar structure as 1 (Figure 1 and Table 1). The E-configuration for both trisubstituted olefins was deduced by the presence of the NOE correlations between H_3-23 (δ_H 1.32, s) and H_3-24 (δ_H 1.77, s), H_3-20 (δ_H 1.58, d, J = 6.6 Hz) and H_3-29 (δ_H 1.50, s), and the absence of the NOE between H-7 (δ_H 5.28, s) and H_3-24 (Figure 2).

The strong NOE correlations among H-9 (δ_H 3.03, s), H-15 (δ_H 2.68, s), and H_3-25 (δ_H 1.13, s) indicated that the bicyclo[4.2.0]octadiene unit in 2 has the same relative stereochemistry as that of 1 (Figure 2). Then, it was suggested that compound (2) might be a stereo-isomer of 1 at the C-17 secondary hydroxy function, since slight differences of the NMR spectra were observed at C-9 (1: δ_H 2.68 s, δ_C 60.7; 2: δ_H 3.03 s, δ_C 58.8), C-15 (1: δ_H 2.39 s, δ_C 51.7; 2: δ_H 2.68 s, δ_C 47.5), C-16 (1: δ_C 49.6; 2: δ_C 48.6), C-17 (1: δ_C 49.8; 2: δ_C 50.6), C-18 (1: δ_C 139.0; 2: δ_C 139.0), C-19 (1: δ_C 129.1; 2: δ_C 129.1), C-20 (1: δ_C 149.1; 2: δ_C 149.1), C-21 (1: δ_C 28.5; 2: δ_C 28.5).
δ$_H$ 3.84 s, δ$_C$ 86.3; 2: δ$_H$ 3.66 s, δ$_C$ 85.4), C-18 (1: δ$_C$ 136.5; 2: δ$_C$ 136.2), C-29 (1: δ$_H$ 1.55 s, δ$_C$ 14.4; 2: δ$_H$ 1.50 s, δ$_C$ 12.6)-signals. The Dess-Martin periodinane treatment of 1 and 2 afforded 17-keto derivatives (3) and (4), respectively. Although the NMR spectra of 3 and 4 were also highly similar, 3 and 4 were not identical by HPLC analysis. Then, an intensive study was required to elucidate the stereostructure of 2.

Figure 1. COSY and key HMBC correlations for shimalactone B (2)

Firstly, the relative stereostructure of the oxabicyclo[2.2.1]heptane unit in 2 was proposed as b or c in Figure 2 on the basis of its NOE correlations. Compound (2) was treated with NaBH$_4$ in Et$_2$O to give two reduced products (6) and (8) (Figure 3). Comparison of the $^1$H NMR spectra of 6 and 8 with 5 and 7,
which were produced from 1 by the same NaBH₄ treatment,⁵ revealed that the relative stereostructure of the oxabicyclo[2.2.1]heptane unit in 6 and 8 were identical with those of 5 and 7, respectively. Then, the relative stereostructure of the oxabicyclo[2.2.1]heptane unit in 2 was clarified to be the same as that of 1.

Table 1. ¹H (600 MHz) and ¹³C (150 MHz) NMR spectral data for shimalactone B (2) in CDCl₃.

<table>
<thead>
<tr>
<th>No.</th>
<th>¹³C δ C</th>
<th>¹H δ H (mult., J (Hz))</th>
<th>HMBC correlations a)</th>
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<tr>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>58.2</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
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<td></td>
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<td>4</td>
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<td>C-3, C-22</td>
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<td>5.12 (1H, d, 1.9)</td>
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<td>6</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>123.3</td>
<td>5.28 (1H, s)</td>
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<td>8</td>
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<tr>
<td>9</td>
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<td>3.03 (1H, s)</td>
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<td>2.68 (1H, s)</td>
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<td>C-14, C-15</td>
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<td>C-15, C-16, C-17</td>
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<td>29</td>
<td>12.6</td>
<td>1.50 (3H, s)</td>
<td>C-18, C-19</td>
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a) C coupled with H.

Secondly, the CD spectra of 1 and 2 showed a complex and different pattern from each other (Figures 4, 5). Compounds (7) and (8) lacking a 5-membered ketone chromophore also showed complex and different CD spectra, respectively. The differential CD spectrum of 1 and 7 showed a strong negative maximum at 231 nm and a weak negative maximum at 284 nm (Figure 4), while that of 2 and 8 showed a strong negative maximum at 245 nm and a weak positive maximum at 288 nm (Figure 5). The CD maxima at around 240 nm of the differential CD spectra were presumed to come mainly from the interaction between the five-membered ketone and the lactone carbonyl chromophores, and the CD maxima at around 285 nm were presumed to come from the interaction between the five-membered
ketone and the homoannular diene chromophores. Based on the above information, we tentatively presumed that the absolute configuration of the oxabicyclo[2.2.1]heptane unit of 2 is the same as that of 1, while the absolute configuration of the bicyclo[4.2.0]octadiene unit of 2 might be different from that of 1.

![Diagram of molecular structures and NMR spectra](image)

**Figure 3.** $^1$H NMR spectral data for the oxabicyclo[2.2.1]heptane units in 5, 6, 7 and 8

![Graphs of CD and UV spectra](image)

**Figure 4.** CD and UV spectra of shimalactone A (1, solid line) and 7 (dotted line) and differential CD spectrum (bold line) of 1 and 7 in methanol

**Figure 5.** CD and UV spectra of shimalactone B (2, solid line) and 8 (dotted line) and differential CD spectrum (bold line) of 2 and 8 in methanol
Thirdly, the ozonolysis followed by 2,4-dinitrophenylhydrazine treatment of 1 and 2 respectively afforded hydrazone derivatives (9 and 10), which were found by spectral analysis to be identical including the optical rotation (Figure 6). This result confirmed that the absolute configuration for the oxabicyclo[2.2.1]heptane unit of 2 was the same as that of 1. Then, the absolute configuration of the bicyclo[4.2.0]octadiene unit of 2 should be opposite to that of 1, since 3 and 4 are different.

\[
\text{shimalactone A (1)} \quad \text{or} \quad \text{shimalactone B (2)}
\]

\[
\begin{align*}
\text{i) } & \text{O}_3, \text{CH}_2\text{Cl}_2, \text{rt;} \quad \text{ii) } \text{Me}_2\text{S, rt;} \\
& \text{iii) } 2,4\text{-dinitrophenylhydrazine, AcOH}
\end{align*}
\]

Figure 6. Hydrazone derivatives prepared from shimalactones A (1) and B (2)

Figure 7. CD and UV spectra of shimalactone B (2, solid line) and 11 (dotted line) and differential CD spectrum (bold line) of 11 and 2 in methanol

To determine the absolute configuration at the C-17 secondary hydroxy group in 2, the pyridine solution of 2 was treated with benzoyl chloride and 4-(dimethylamino)pyridine (DMAP) at 80°C to give the
benzoate (11). The differential CD spectrum of 11 and 2 showed two positive maxima at 228 nm (Δε = +6.1) and 271 nm (+4.4). The CD maximum at 228 nm can be attributed to the interaction (plus chirality) between the benzoate and the Δ^18 olefin chromophores, suggesting the R configuration at C-17 in 11. On the other hand, the CD maximum at 271 nm, which was not observed in the differential CD spectrum between 1 and its benzoate derivative, is presumed to come from the interaction between the benzoate and the homoannular diene chromophores in 11 (Figure 7).

The absolute configuration at C-17 in 2 was further confirmed by application of the modified Mosher’s method. Thus, a pyridine solution of 2 was treated with DMAP, (S)-(+-) or (R)-(−)-methoxy(trifluoromethyl)phenylacetyl chloride [(S)- or (R)-MTPA chloride] at 80 °C to obtain two Mosher esters (12a) and (12b). The detailed NMR spectral analyses of the 17-MTPA esters (12a) and (12b) supported that the absolute configuration at C-17 in 2 is R (Figure 8). Consequently, it was clarified that shimalactone B (2) is an enantiomer of the bicyclo[4.2.0]octadiene unit in shimalactone A (1).

Shimalactones A (1) and B (2) are polyketides having two bicyclic ring systems (bicyclo[4.2.0]octadiene unit and oxabicyclo[2.2.1]heptane unit). Recently, two similar polyketides, SNF4435C (16) and SNF4435D (17), having a bicyclo[4.2.0]octadiene unit have been found as immunosuppressive substances from the cultured broth of Streptomyces spectabilis. The biomimetic synthesis of the bicyclo[4.2.0]octadiene unit has been also achieved from an all-trans tetraene precursor through a tandem double electrocyclization. Shimalactones A (1) and B (2) are presumed to be biosynthesized from an acetate and nine propionates. Thus, a polyene lactone (14) is formed from a precursor (13).
conrotatory 8π-electrocyclization of the common precursor (14) affords cyclooctatrienes (15a) and (15b), which are stereoselectively converted to 1 and 2 by disrotatory 6π-electrocyclization (Scheme 1).9,10 The absolute configurations of SNF4435C (16) and SNF4435D (17) have not yet been elucidated. On the basis of the structure-relationship of shimalactones A and B, SNF4435C (16) and SNF4435D (17) might be enantiomers of the bicyclo[4.2.0]octadiene unit. Shimalactones A (1) and B (2) induced neuritogenesis in neuroblastoma Neuro 2A cells at the concentration of 10 µg/mL. At the higher concentration of 20 µg/mL, they were cytotoxic against Neuro 2A cells.

EXPERIMENTAL

Optical rotations were measured using a JASCO DIP-370 polarimeter. UV spectra were performed with a Shimazu UV-2425 spectrophotometer. CD spectra were recorded on a JASCO J-720 spectrometer (L = 1 mm). IR spectra were recorded on a JASCO FT/IR-5300 infrared spectrophotometer. NMR spectra were recorded on a Varian Unity Inova 600 (600 MHz for 1H and 150 MHz for 13C) spectrometer. HRFABMS spectra were obtained on a JEOL JMS SX-102 mass spectrometer. Silica gel (Merck 60-230 mesh) and precoated thin-layer chromatography (TLC) plates (Merck, Kiesel gel, 60F254) were used for column chromatography and TLC. Spots on TLC were detected by spraying 1%Ce(SO4)2/10%H2SO4 with subsequent heating. Artificial seawater was prepared by Aquamarine (Yashima Pure Chemical Co. LTD, Japan).

Fungus material, culture conditions, and extraction. The Emericella variecolor GF10 strain was separated from the marine sediment collected from a depth of 70 m off Gokasyo Gulf, Mie Prefecture, Japan, in 2002 and deposited in our laboratory.4 The GF10 strain was cultured in the MG medium (malt extract: 20 g, glucose: 20 g, bact peptone: 1 g, artificial seawater: 1000 mL) for 5 days at 30 °C. Then, the broth of the strain was inoculated into rice solid medium (rice: 25 g, artificial seawater: 50 mL, in a 500 mL flask) and cultured under static conditions for 2 weeks at 30 °C. The culture was extracted with acetone and a mixed solvent (EtOAc-MeOH-acetone= 1:2:4), and then the organic solvent was combined and evaporated under reduced pressure to give an extract. The extract was partitioned into a 2-butane-H2O mixture, and the 2-butane-soluble portion (12.0 g) was further partitioned into an n-hexane-90% aq. MeOH mixture to furnish a MeOH extract (5.0 g).

Isolation of shimalactone B (2). The MeOH extract (5.0 g) of the solid culture (75 g x 40) was fractionated by SiO2 column chromatography (n-hexane-EtOAc) to give seven fractions (A-G). Fraction B (460 mg) was then subjected to SiO2 column chromatography (CHCl3-MeOH) to afford four fractions (B-1 to B-4). Fraction B-3 (183 mg) was separated by reversed phase HPLC (Cosmosil 5C18-AR,
MeOH-H2O = 80:20) to obtain three fractions B-3.1, B-3.2 and B-3.3. Fraction B-3.3 (112 mg) was further purified by HPLC (Cosmosil 5SL, n-hexane-EtOAc = 88:12) to furnish shimalactone A (I, 77 mg) and shimalactone B (2, 25 mg).

Shimalactone B (2) was obtained as colorless oil: [α]23\text{D}^{−} = −48° (c 1.50, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.15), 281 (3.57) nm; CD (MeOH) Δε_{207} +2.1, Δε_{213} 0, Δε_{225} -5.2 (sh), Δε_{244} -9.8, Δε_{307} 0, Δε_{324} 0.5; IR (KBr) ν_{max} 3510, 2975, 1795, 1750 cm⁻¹; \textsuperscript{1}H and \textsuperscript{13}C NMR, see Table 4; FABMS m/z 475 [M+Na]+; HRFABMS m/z 475.2815 (calcd for C_{29}H_{40}O_{4}Na, 475.2824).

Dess-Martin oxidation of shimalactones A (1) and B (2). A CH_{2}Cl_{2} (1.5 mL) solution of I (6 mg) was treated with Dess-Martin periodinane (15 mg) and the mixture was stirred for 1 h at rt. The reaction mixture was poured into 10 mL of saturated aq. NaHCO_{3} solution then extracted with EtOAc. The EtOAc phase was washed with brine and dried over anhydrous MgSO_{4}. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by SiO_{2} gel chromatography (eluted with n-hexane-EtOAc = 1 : 1) to give 3 (6 mg). Treatment of 2 (6 mg) by the same procedure as described above afforded 4 (5 mg). When they were analyzed by HPLC (Cosmosil 5SL, 10 mm x 250 mm; mobile phase, n-hexane-EtOAc = 5:1; flow rate, 4.0 mL/min), 3 (t_{R} 8.2 min) and 4 (t_{R} 8.6 min) displayed different retention time.

3: colorless amorphous; \textsuperscript{1}H NMR (CDCl_{3}, 600 MHz) δ_{H} 6.20 (1H, q, J = 6.9 Hz, H-19), 5.55 (1H, s, H-13), 5.38 (1H, s, H-7), 5.18 (1H, d, J = 1.9 Hz, H-5), 4.72 (1H, s, H-11), 3.03 (1H, s, H-15), 2.83 (1H, s, H-9), 2.65 (1H, dq, J = 1.9, 7.1 Hz, H-4), 1.82 (3H, s, H_{3}-24), 1.77 (3H, d, J = 6.9 Hz, H_{3}-20), 1.74 (3H, s, H_{3}-29), 1.72 (3H, s, H_{3}-27), 1.69 (3H, s, H_{3}-26), 1.41 (3H, s, H_{3}-23), 1.30 (3H, s, H_{3}-28), 1.25 (3H, d, J = 7.1 Hz, H_{3}-22), 1.18 (3H, s, H_{3}-21), 1.04 (3H, s, H_{3}-25); \textsuperscript{13}C NMR (CDCl_{3}, 150 MHz) δ_{C} 207.2 (C, C-3), 206.3 (C, C-17), 171.4 (C, C-1), 137.6 (C, C-8), 136.9 (CH, C-19), 134.7 (C, C-18), 132.7 (C, C-14), 130.0 (C, C-12), 126.0 (C, C-7), 123.9 (CH, C-13), 123.7 (CH, C-11), 84.7 (CH, C-5), 70.0 (C, C-6), 61.6 (CH, C-9), 58.2 (C, C-2), 55.2 (C, C-16), 49.6 (CH, C-15), 44.2 (CH, C-4), 41.6 (C, C-10), 30.9 (CH_{3}, C-25), 22.8 (CH_{3}, C-27), 22.1 (CH_{3}, C-26), 20.8 (CH_{3}, C-24), 18.1 (CH_{3}, C-28), 16.9 (CH_{3}, C-23), 14.5 (CH_{3}, C-20), 12.0 (CH_{3}, C-29), 11.6 (CH_{3}, C-22), 4.7 (CH_{3}, C-21). FABMS m/z 473 [M+Na]+; HRFABMS m/z 473.2675 (calcd for C_{29}H_{38}O_{4}Na, 473.2668).

4: colorless amorphous; \textsuperscript{1}H NMR (CDCl_{3}, 600 MHz) δ_{H} 6.31 (1H, q, J = 6.9 Hz, 19-H), 5.54 (1H, s, H-13), 5.50 (1H, s, H-7), 5.17 (1H, d, J = 1.9 Hz, H-5), 4.60 (1H, s, H-11), 3.02 (1H, s, H-15), 2.80 (1H, s, H-9), 2.75 (1H, dq, J = 1.9, 7.1 Hz, H-4), 1.80 (3H, s, H_{3}-24), 1.77 (3H, d, J = 6.9 Hz, H_{3}-20), 1.74 (3H, s, H_{3}-29), 1.72 (3H, s, H_{3}-27), 1.69 (3H, s, H_{3}-26), 1.40 (3H, s, H_{3}-23), 1.34 (3H, s, H_{3}-28), 1.26 (3H, d, J =
7.1 Hz, H$_2$-22), 1.18 (3H, s, H$_3$-21), 1.06 (3H, s, H$_3$-25); $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta_c$ 207.0 (C, C-3), 206.3 (C, C-17), 171.3 (C, C-1), 137.5 (C, C-8), 137.0 (CH, C-19), 134.7 (C, C-18), 132.8 (C, C-14), 130.6 (C, C-12), 125.7 (CH, C-7), 124.1 (CH, C-13), 123.2 (CH, C-11), 85.1 (CH, C-5), 70.0 (C, C-6), 61.0 (CH, C-9), 58.3 (C, C-2), 55.0 (C, C-16), 49.6 (CH, C-15), 44.2 (CH, C-4), 41.8 (C, C-10), 31.1 (CH$_3$, C-25), 22.8 (CH$_3$, C-27), 21.9 (CH$_3$, C-26), 21.6 (CH$_3$, C-24), 18.0 (CH$_3$, C-28), 16.9 (CH$_3$, C-23), 14.5 (CH$_3$, C-20), 12.0 (CH$_3$, C-29), 11.6 (CH$_3$, C-22), 4.7 (CH$_3$, C-21); FABMS m/z 473 [M+Na]$^+$; HRFABMS m/z 473.2657 (calcd for C$_{29}$H$_{38}$O$_4$Na, 473.2668).

Reduction of shimalactones A (1) and B (2). An Et$_2$O (10 mL) solution of 1 (21 mg) was treated with NaBH$_4$ (5 mg) for 4 days at rt. The reaction mixture was filtered, and the filtrate was purified by HPLC (Cosmosil 5SL, n-hexane-EtOAc= 4:1) to give 5 (9 mg) and 7 (7 mg). The treatment of 2 (20 mg) with the same procedure afforded 6 (9 mg) and 8 (7 mg).

6: colorless amorphous; $^1$H NMR (CDCl$_3$, 600 MHz) $\delta_H$ 5.51 (1H, s, H-7), 5.50 (1H, q, $J = 6.7$ Hz, H-19), 5.45 (1H, s, H-13), 4.92 (1H, s, H-11), 4.69 (1H, brs, H-5), 3.89 (1H, s, H-17), 3.24 (1H, d, $J = 3.0$ Hz, H-3), 2.90 (1H, s, H-9), 2.47 (1H, s, H-15), 2.25 (1H, m, H-4), 1.75 (3H, s, H$_3$-24), 1.66 (3H, s, H$_3$-26), 1.57 (3H, d, $J = 6.7$ Hz, H$_3$-20), 1.56 (3H, s, H$_3$-29), 1.48 (3H, s, H$_3$-27), 1.18 (3H, d, $J = 7.2$ Hz, H$_3$-22), 1.15 (3H, s, H$_3$-21), 1.12 (3H, s, H$_3$-25), 1.09 (3H, s, H$_3$-23), 1.08 (3H, s, H$_3$-28); $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta_C$ 178.3 (C, C-1), 136.8 (C, C-8), 135.8 (C, C-18), 132.4 (C, C-14), 129.2 (C, C-12), 127.3 (CH, C-7), 124.3 (CH, C-11), 124.2 (CH, C-19), 123.7 (CH, C-13), 88.4 (CH, C-5), 88.3 (CH, C-17), 80.0 (CH, C-3), 64.8 (CH, C-9), 59.9 (C, C-6), 56.2 (C, C-2), 50.2 (C, C-16), 48.4 (CH, C-15), 45.0 (CH, C-4), 41.3 (C, C-10), 31.6 (CH$_3$, C-25), 23.5 (CH$_3$, C-27), 22.0 (CH$_3$, C-26), 18.5 (CH$_3$, C-24), 16.4 (CH$_3$, C-23), 14.5 (CH$_3$, C-22), 14.2 (CH$_3$, C-28), 13.3 (CH$_3$, C-29), 13.0 (CH$_3$, C-20), 6.5 (CH$_3$, C-21); FABMS m/z 477 [M+Na]$^+$; HRFABMS m/z 477.2960 (calcd for C$_{29}$H$_{42}$O$_4$Na, 477.2981).

8: colorless amorphous; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 202 (4.20), 281 (3.60) nm; CD (MeOH) $\Delta\varepsilon_{202}$ 3.5, $\Delta\varepsilon_{215}$ 0, $\Delta\varepsilon_{228}$ -7.8, $\Delta\varepsilon_{248}$ -1.7, $\Delta\varepsilon_{281}$ -3.5; $^1$H NMR (CDCl$_3$, 600 MHz) $\delta_H$ 5.47 (1H, s, H-13), 5.42 (1H, q, $J = 6.7$ Hz, H-19), 5.31 (1H, s, H-7), 4.85 (1H, s, H-11), 4.63 (1H, s, H-5), 4.05 (1H, d, $J = 9.1$ Hz, H-3), 3.66 (1H, s, H-17), 3.04 (1H, s, H-9), 2.71 (1H, s, H-15), 2.57 (1H, dq, $J = 9.1, 7.2$ Hz, H-4), 1.66 (3H, s, H$_3$-24), 1.65 (3H, s, H$_3$-26), 1.58 (3H, d, $J = 6.7$ Hz, H$_3$-20), 1.57 (3H, s, H$_3$-27), 1.51 (3H, s, H$_3$-29), 1.15 (3H, s, H$_3$-21), 1.13 (3H, s, H$_3$-25), 1.09 (3H, s, H$_3$-23), 1.07 (3H, s, H$_3$-28), 1.03 (3H, d, $J = 7.2$ Hz, H$_3$-22); $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta_C$ 177.0 (C, C-1), 137.2 (C, C-8), 136.3 (C, C-18), 132.4 (C, C-14), 129.7 (C, C-12), 124.5 (CH, C-7), 124.1 (CH, C-11), 123.6 (CH, C-19), 123.5 (CH, C-13), 87.0 (CH, C-5), 85.3 (CH, C-17), 74.8 (CH, C-3), 61.0 (C, C-6), 58.7 (CH, C-9), 54.3 (C, C-2), 48.8 (C, C-16), 47.4 (CH, C-15), 41.1 (C, C-10), 36.2 (CH, C-4), 30.8 (CH$_3$, C-25), 23.5 (CH$_3$, C-27), 22.1 (CH$_3$, C-26), 19.5
(CH₃, C-24), 16.2 (CH₃, C-23), 16.0 (CH₃, C-28), 13.0 (CH₃, C-20), 12.5 (CH₃, C-29), 9.1 (CH₃, C-22), 7.9 (CH₃, C-21); FABMS m/z 477 [M+Na]+; HRFABMS m/z 477.2999 (calcd for C₂₉H₄₂O₄Na, 477.2981).

**Preparation of Hydrazone Derivatives 9 and 10.** A CH₂Cl₂ (10 mL) solution of shimalactone A (1, 16 mg) was treated with ozone (O₃) for 20 min at rt, and then (CH₃)₂S (0.1 mL) was added. After stirring for 2 h at rt, the mixture was treated with 2,4-dinitrophenylhydrazine (20 mg) and AcOH (0.1 mL) and further stirred for 1 h at rt. The reaction mixture was poured into saturated aq. NaHCO₃ solution and then extracted twice with EtOAc. The EtOAc phase was washed with brine and dried over anhydrous MgSO₄. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by SiO₂ gel chromatography (n-hexane-EtOAc = 1 : 1) to give 9 (7 mg). Shimalactone B (2, 12 mg) was treated by the same procedure to afford 10 (4 mg).

**9:** yellow amorphous; [α]°D -85° (c 0.51, MeOH); ¹H NMR (CDCl₃, 600 MHz) δH 11.10 (1H, s), 9.10 (1H, d, J = 2.3 Hz), 8.40 (1H, dd, J = 9.5, 2.3 Hz), 7.61 (1H, d, J = 9.5 Hz), 7.35 (1H, s), 4.92 (1H, d, J = 2.2 Hz), 2.72 (1H, dq, J = 2.2, 7.2 Hz), 1.53 (3H, s), 1.37 (3H, s), 1.26 (3H, d, J = 7.2 Hz); ¹³C NMR (CDCl₃, 150 MHz) δC 205.4, 170.6, 145.6 (C x 2), 144.3, 130.7, 123.1, 116.5, 83.0, 68.4, 60.4, 59.5, 43.9, 15.9, 11.4, 5.9; FABMS m/z 399 [M+Na]+; HRFABMS m/z 399.0932 (calcd for C₁₆H₁₆N₄O₇Na, 399.0917).

**10:** yellow amorphous; [α]°D -84° (c 0.28, MeOH).

**Benzylation of shimalactone B** (2). A solution of 2 (5 mg) in pyridine (1.0 mL) was treated with benzyol chloride (0.1 mL) and DMAP (2.0 mg) and the mixture was stirred for 2 h at 80 °C. The reaction was quenched by saturated aq. NH₄Cl and extracted with EtOAc. After being washed with saturated aq. NH₄Cl, saturated aq. NaHCO₃, and brine in sequence, the EtOAc layer was dried over MgSO₄ and evaporated in vacuo to afford a crude product. The crude product was purified by reversed phase HPLC (Cosmosil 5C18-AR, MeOH-H₂O = 80:20) to obtain 11 (5 mg).

**11:** colorless oil; IR (KBr) νmax 2970, 1795, 1750, 1720 cm⁻¹; UV (MeOH) λmax (log ε) 201 (4.39), 230 (4.22), 280 (3.62); CD (MeOH) Δε₂₁₃ -2.5, Δε₂₂₄ 0, Δε₂₂₇ 0.6, Δε₂₃₀ -6.4, Δε₂₆₄ 0, Δε₂₈₀ 1.5; ¹H NMR (CD₃OD, 600 MHz) δH 8.04 (2H, d, J = 7.7 Hz, H-2’, 6’), 7.62 (1H, t, J = 7.7 Hz, H-4’), 7.50 (2H, t, J = 7.7 Hz, H-3’, 5’), 5.65 (1H, q, J = 6.9 Hz, H-19), 5.50 (1H, s, H-13), 5.29 (1H, s, H-5), 5.27 (1H, s, H-7), 5.25 (1H, s, H-17), 4.85 (1H, s, H-11), 3.04 (1H, s, H-9), 2.77 (1H, dq, J = 2.0, 7.4 Hz, H-4), 2.50 (1H, s, H-15), 1.72 (3H, s, H₂-24), 1.64 (6H, s, H₂-26, 29), 1.61 (3H, d, J = 6.9 Hz, H₃-20), 1.55 (3H, s, H₃-27), 1.19 (3H, s, H₃-28), 1.14 (3H, s, H₃-25), 1.11 (3H, s, H₃-23), 1.11 (3H, d, J = 7.4 Hz, H₃-22), 1.03
(3H, s, H₃-21); ¹H NMR (CDCl₃, 600 MHz) δ 8.03 (2H, dd, J = 7.4, 1.0 Hz, H-2’, 6’), 7.56 (1H, td, J = 7.4, 1.0 Hz, H-4’), 7.44 (2H, t, J = 7.4 Hz, H-3’, 5’), 5.63 (1H, q, J = 6.6 Hz, H-19), 5.47 (1H, s, H-13), 5.28 (1H, s, H-7), 5.22 (1H, s, H-17), 5.04 (1H, d, J = 1.8 Hz, H-5), 4.74 (1H, s, H-11), 2.97 (1H, s, H-9), 2.71 (1H, dq, J = 1.8, 7.4 Hz, H-4), 2.47 (1H, s, H-15), 1.66 (3H, s, H₃-24), 1.64 (3H, s, H₃-26), 1.60 (3H, s, H₃-29), 1.57 (3H, d, J = 6.6 Hz, H₃-20), 1.53 (3H, s, H₃-27), 1.15 (3H, s, H₃-28), 1.15 (3H, d, J = 7.4 Hz, H₃-22), 1.14 (3H, s, H₃-25), 1.12 (3H, s, H₃-23), 1.07 (3H, s, H₃-21); ¹³C NMR (CDCl₃, 150 MHz) δ C 207.4 (C, C-3), 171.6 (C, C-1), 165.5 (C, C-7’), 138.5 (C, C-8), 133.1 (CH, C-4’), 132.1 (C, C-18), 131.5 (C, C-14), 130.6 (C, C-12), 130.6 (C, C-1’), 129.5 (CH, C-2’, 6’), 128.5 (CH, C-3’, 5’), 126.1 (CH, C-19), 124.2 (CH, C-13), 123.2 (CH, C-11), 122.9 (CH, C-7), 87.6 (CH, C-17), 85.3 (CH, C-5), 70.1 (C, C-6), 61.3 (CH, C-9), 58.1 (C, C-2), 48.7 (CH, C-15), 48.6 (C, C-16), 44.0 (CH, C-4), 41.4 (C, C-10), 31.6 (CH₃, C-25), 23.4 (CH₃, C-27), 21.9 (CH₃, C-26), 20.3 (CH₃, C-24), 16.1 (CH₃, C-23), 15.2 (CH₃, C-28), 13.5 (CH₃, C-29), 13.1 (CH₃, C-20), 11.5 (CH₃, C-22), 4.8 (CH₃, C-21); FABMS m/z 579 [M+Na]+; HRFABMS m/z 579.3066 [M+Na]+; (caled for C₃₆H₄₃O₄Na, 579.3086).

Preparation of (R)- or (S)-MTPA ester of shimalactone B (2). A dried pyridine (1 mL) solution of 2 (5 mg) was treated with (S)-MTPA chloride (10 µL), DMAP (2 mg). The reaction mixture was stirred for 2 h at 80 °C. The reaction was quenched by 5% HCl and the whole was extracted with EtOAc. The EtOAc phase was washed by saturated aq. NaHCO₃, brine and then dried over MgSO₄. After evaporation of the solvent, the resulting residue was purified by SiO₂ gel column (n-hexane-EtOAc = 4:1) to afford the (R)-MTPA ester 12a (4.5 mg). A dried pyridine (1 mL) solution of 2 (5 mg) was similarly treated with (R)-MTPA chloride (10 µL), and DMAP (2.0 mg) to afford the (S)-MTPA ester 12b (4 mg).

**12a:** ¹H NMR (CDCl₃, 600 MHz) δH 7.51 (2H, d, J = 7.2 Hz, 2’, 6’-H), 7.40 (3H, m, H-3’, 4’, 5’), 5.48 (1H, s, H-13), 5.45 (1H, q, J = 6.6 Hz, H-19), 5.17 (1H, s, H-7), 5.08 (1H, d, J = 2.2 Hz, H-5), 4.85 (1H, s, H-17), 4.71 (1H, s, H-11), 3.49 (3H, s, OCH₃), 2.89 (1H, s, H-9), 2.71 (1H, dq, J = 2.2, 7.2 Hz, H-4), 2.37 (1H, s, H-15), 1.68 (3H, s, H₃-24), 1.65 (3H, s, H₃-26), 1.57 (3H, d, J = 6.6 Hz, H₃-20), 1.54 (3H, s, H₃-27), 1.42 (3H, s, H₃-29), 1.26 (3H, s, H₃-25), 1.19 (3H, d, J = 7.2 Hz, H₃-22), 1.11 (3H, s, H₃-21), 1.05 (3H, s, H₃-28), 0.96 (3H, s, H₃-25); FABMS m/z 691 [M+Na]+; HRFABMS m/z 691.3251 (caled for C₃₉H₄₁O₆Na, 691.3222).

**12b:** ¹H NMR (CDCl₃, 600 MHz) δH 7.51 (2H, d, J = 7.2 Hz, H-2’, 6’), 7.38 (3H, m, H-3’, 4’, 5’), 5.54 (1H, q, J = 6.6 Hz, H-19), 5.46 (1H, s, H-13), 5.17 (1H, s, H-7), 5.08 (1H, d, J = 2.2 Hz, H-5), 4.94 (1H, s, H-17), 4.67 (1H, s, H-11), 3.55 (3H, s, OCH₃), 2.91 (1H, s, H-9), 2.71 (1H, dq, J = 2.2, 7.2 Hz, H-4), 2.22 (1H, s, H-15), 1.68 (3H, s, H₃-24), 1.63 (3H, s, H₃-26), 1.59 (3H, d, J = 6.6 Hz, H₃-20), 1.55 (3H, s, H₃-27), 1.46 (3H, s, H₃-29), 1.28 (3H, s, H₃-23), 1.20 (3H, d, J = 7.2 Hz, H₃-22), 1.12 (3H, s, H₃-21), 1.02
(3H, s, H-28), 0.83 (3H, s, H-25); FABMS m/z 691 [M+Na]+; HRFABMS m/z 691.3207 (calcld for C_{39}H_{47}O_{6}F_{3}Na, 691.3222).

**Assay for neuritogenic activity against Neuro 2A cells.** Neuro 2A cells were grown in Dulbecco’s modified essential medium (DMEM) with 10% fetal bovine serum (FBS). The cells were kept in incubator at 37 °C with 5% CO_2. The cells were plated on 24-well plates at a density of 2 x 10^4 per well with 1 mL of culture medium. After 24 h cultivation, the medium was exchanged with fresh medium, and the testing sample as 10 µL of EtOH solution was added to each well. After 24 h incubation, morphological changes in the cells were observed under microscope.

**ACKNOWLEDGEMENTS**

The authors are grateful to Prof. Nobuyuki Harada, Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, for the helpful discussion about CD analysis. We are grateful to the Takeda Science Foundation and the Ministry of Education, Culture, Sports, Science, and Technology of Japan for financial support.

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